Applicants' attorney has brought this matter to the attention of the Examiner in a telephone conference held on April 24, 2002, during which the Examiner indicated that the finality of the Action would be withdrawn in writing. Since Applicants have not received anything to that effect from the Patent Office, and to avoid extension of time fees, Applicants are responding to the Action prior to receiving any communication on this matter.

Nonetheless, the removal of the finality of the Official Action appears to be in order and is respectfully requested. In order for the Office to address this issue as soon as possible, Applicants are filing the request for the withdrawal of finality separately via facsimile.

IN THE CLAIMS:

Please amend the claims as shown at the replacement pages attached hereto. The changes made by the amendment are shown in Attachment I.

REMARKS

Reconsideration of the application is respectfully requested.

Claims 120-224 have been amended to recite the structural formula of the claimed compounds and the type of interflavan linkages between the monomeric units in a cocoa procyanidin oligomer. Support may be found in the application under "Detailed Description," pages 7-9. Claims 171-179 and 186-193 have been amended to recite "NO-dependent hypertension" rather than "hypertension" to reflect that cocoa procyanidin monomers and/or oligomers act on hypertension through NO, as a mechanism. Support for this amendment is found under "NO-associated Utilities," pages 15-16. Thus, no new matter has been added.

Allowable Subject Matter

Applicants acknowledge the Examiner's finding of the allowable subject matter of claims 141-143 and 180-185.

Because the claims indicated allowable by the Examiner recite a combination of a cocoa procyanidin monomer and/or oligomer and a cyclooxygenase modulator, Applicant believe that claims 129-131; 202-204; and 213-215 should also be allowable on that ground. An action to that effect is respectfully requested.

Rejection under 35 USC Section 103

Claims 120-140 and 144-179 stand rejected as obvious over Clapperton *et al.* The Examiner states that Clapperton discloses compositions comprising polyphenols (procyanidins) from cocoa, which compositions are made into liquor. The Examiner acknowledges that Clapperton fails to teach the compositions in packages with instructions for use but states that these are not patentable distinctions since a person of skill in the art would have been motivated to make additional compositions by packaging the compositions disclosed by Clapperton with instructions on methods of use.

As noted above, because claims 141-143 and 180-185 (drawn to the combination of a cocoa procyanidin monomer and/or oligomer with a cyclooxygenase inhibitor) were indicated allowable, Applicants believe that claims 129-131 should be allowable for the same reason. As to the rejection of claims 120-128, 132-141, and 144-179, Applicants respectfully traverse.

Claims 120-128, 132-141, and 144-179 recite an article of manufacture, a package, comprising a composition and instructions for use. The composition comprises a cocoa procyanidin monomer and/or oligomer in combination with a carrier. The carrier is a pharmaceutical, veterinary, dietary supplement or food carrier, *i.e.*, a carrier designed for delivery of the cocoa procyanidin monomer and/or oligomer to a subject in need thereof. The instructions for use functionally define (and thus limit) the composition as a *therapeutic* composition, *i.e,* the composition that, when administered, has a therapeutic effect. In other words, the recitation of the instruction for use functionally defines the amount of cocoa procyanidin monomer and/or oligomer as the amount effective for the treatment recited in the claims.

Clapperton fails to suggest any article of manufacture comprised of a *therapeutic* composition and instructions for therapeutic use. Clapperton teaches that cocoa beans can be dehusked and milled into liquor and that defatted cocoa powder may be used to make extracts. He also states that he isolated various procyanidin fractions (although the isolation procedure is not completely described, *see* page 2, under "Isolation of Procyanidins"). However, Applicants' claims do not cover any of the compositions disclosed in Clapperton. The compositions covered here contain therapeutically effective amount of cocoa procyanidin monomer and/or oligomer; they contain an appropriate carrier suitable for therapeutic delivery; and they are combined with instructions for therapeutic use.

The Examiner states that a person of skill in the art would have been motivated to make an "additional composition comprising polyphenol (procyanidin)" (Official Action, page 3, first par.) and that "having composition in packages with instructions on methods of use is well known in the art (Official Action, sentence spanning pages 2-3). But, Applicants do not claim packages generally; they claim packages comprising a particular therapeutic composition. Clapperton does not teach or suggest that cocoa procyanidin monomers and/or oligomers have therapeutic effects and, therefore, cannot suggest manufacturing delivery vehicles with cocoa procyanidin monomers and/or oligomers for administration to humans or animals, nor can he suggest the therapeutically effective amounts that such compositions must contain. All that Clapperton teaches is that cocoa procyanidins are bitter and astringent. Without suggestion of any therapeutic effects of these compounds by Clapperton, a person of skill in the art would *not* have been motivated to combine the compounds with carriers adapted for therapeutic applications and prepare articles of manufacture claimed herein. In fact, due to the compounds' unpleasant taste, a person of skill in the art would have avoided adding the compounds into any composition, e.g. food, intended for human or animal consumption.

Moreover, other prior art cited in the Official Action, Lunder and Ho, would have discouraged a person of skill in the art even from testing the procyanidin compounds disclosed by Clapperton for any therapeutic use since, as discussed in more detail below, Lunder and Ho teach that gallated compounds are those that may cause beneficial health effects. Furthermore, the articles of manufacture recited in claims 120-140 and 144-179 comprise compositions having a series of unexpected health benefits (e.g. non-steroidal drug effect, NO synthesis induction), which are discussed in more detail below with reference to the method claims.

In view of the above, the invention of claims 120-140 and 144-179, *as a whole,* would *not* have been obvious to a person of skill in the art as of the effective filing date. Applicants, therefore, believe that the withdrawal of the Clapperton rejection is in order. Such action is respectfully requested.

* * *

Method claims 186-224 stand rejected as obvious over Lunder in view of Chi-Tang Ho. Lunder is cited as teaching the use of green tea polyphenols for anti-inflammatory, anti-hypertensive, anti-arteriosclerosis and anti-oxidant effects; and Ho is cited as teaching procyanidin derivatives and that they retard lipid oxidation. The Examiner states that it would

have been obvious to use "the monomers and oligomers as anti-inflammatory, antihypertensive, antiarteriosclerosis, antioxidant, etc. at the time the invention was made" (Official Action, page 4). Applicants respectfully traverse the rejection.

As to claims 202-204 and 213-215, Applicants note that they are independently patentable for the same reason as claim 141-143 and 180-185, which the Examiner indicated allowable.

Applicants believe that it would be helpful to provide some background on the type of compounds that are being claimed here. The rejected method claims call for therapeutic use of *cocoa* procyanidin monomers and/or oligomers, *i.e.*, compounds obtained from cocoa or synthetically prepared to correspond in chemical structures to naturally occurring cocoa procyanidins. As detailed below, cocoa procyanidins represent a *subclass* of all known procyanidins and, thus, the issue is whether the recited therapeutic uses of this particular subclass would have been obvious to a person of skill in the art.

Procyanidins are a large group of compounds that have been identified in a number of plant species, for example, in tea, grapes, and cocoa. The basic building block of these compounds is a three ring, *flavonoid* structure (*see* Attachment II for relevant structures). Referring to Attachment II, depending on the chemical groups and their location on the basic three ring structure, flavonoids may be (i) flavon-3-ols (e.g. quercetin, rutin, kaempferol); and (ii) flavan-3-ols (also generically referred to as catechins). Flavan-3-ols, catechins, or as Applicants refer to them, procyanidin monomers may be: (i) catechin, (ii) epicatechin, (iii) catechin gallate, and (iv) epicatechin gallate; with two additional related, galloylated forms also being present in some plant species: (v) epigallocatechin and (vi) epigallocatechingallate. Referring to Attachment II, galloylated forms contain three (rather than two) –OH groups on ring B, while gallates contain a gallic acid group attached to the carbon at position 3. The presence of more than one type of monomers creates a large number of procyanidin oligomers. For example, an oligomer may contain only epicatechin, only catechin, a mixture of both, or at least one gallated catechin, gallated epicatechin or galloylated forms. Unlike cocoa, tea and grapes (and wines derived therefrom), contain gallated and/or galloylated forms. The present claims have been amended by the insertion of structural formulas to emphasize this distinction.

In view of the above, the proper inquiry in the present case is whether the methods of use of the of procyanidin monomers and/or oligomers recited in claims 186-224 would have been

obvious to a person of skill in the art over the teachings of Lunder in view of Ho. Applicants respectfully submit that this question must be answered in the negative for the reasons detailed below.

Lunder discloses, at pages 116-117 (see text under the subtitle "Physiological Properties"), that "[t]ea is believed to exhibit the following physiological properties" and lists twelve different effects, including the ones recited by the Examiner. Applicants first note that these statements are made with respect to *tea*, which is a *complex mixture* of various chemical compounds many of which are not found in cocoa.

Lunder goes on further to state that "helpful effects [of tea] cannot be ascribed only to the presence of caffeine, but a great importance is given to catechins (flavanols)" (top of page 117). In order to understand what this statement would have suggested to a person of skill in the art, as of the effective filing date of the present application, it is necessary to review the polyphenol content of tea disclosed by Lunder. Referring to Table III (page 116), green tea contains six types of flavanol monomers: epigallocatechin, gallocatechin, epicatechin, catechin, epigallocatechingallate, and epicatechingallate. Only two, epicatechin and catechin, are present in cocoa and are recited in the present claims. These two procyanidin monomers represent minor components of green tea: catechin is present at a concentration of 0.35 g per 100 g of tea leaves and epicatechin is present at 0.63 g/100 g. In contrast, epigallocatechingallate, which is not found in cocoa, is present at a concentration of 10.55 g/100g, representing about 50-60 % of total catechins (page 116, Table III; page 114, Abstract).

Lunder's disclosure does not rest at the above. He further teaches that, from several catechins present in tea, *epigallocatechingallate* is responsible for its antioxidant effects; and shows a good correlation between the antioxidant effect and the epigallocatechingallate content of various teas (*see* pages 118 and 119, Figure 2 and Table V).

In view of Lunder's disclosure that: (i) other tea compounds, such as caffeine, may contribute to its health effects; and (ii) tea anti-oxidant properties are correlated with the presence of epigallocatechingallate, a person of skill in the art would not have been motivated to prepare compositions comprising epicatechin and catechin alone, and/or oligomers based thereon, and utilize them for treating inflammation, atherosclerosis or hypertension with any reasonable expectation of success. In fact, because Lunder states that epigallocatechingallate (rather than any other polyphenolic tea component) should be further studied (*see* page 119,

"Conclusion"), Lunder teaches away from utilizing catechin and epicatechin (and oligomers thereof) recited in the present claims for any health benefit.

The teaching of Ho, when combined with the teaching of Lunder, does not add anything different to the above analysis. Ho also teaches away from the present invention. Thus, Ho tested four tea monomers and four tea dimers in a soybean lypoxygenase assay (*see* page 6, Table I). Referring to Table I, and as explained in the paragraph there above, epigallocatechingallate inhibited lypoxygenase activity, and so did other two gallated forms of epicatechin (epicatechin gallate and epigallocatechin). Two gallated dimers were also effective (*see* Attachment II for structural formulas of theaflavins). In contrast, Ho showed that epicatechin was "relatively inactive" (page 6).

Thus, from the combined teachings of Lunder and Ho, a person of skill in the art would have concluded that the *gallate* group was critical for anti-oxidant and lypoxygenase inhibitory activities, and would therefore not have been motivated to prepare cocoa procyanidin monomers and/or oligomers recited in the present claims (*i.e., non*-gallated compounds) and use them, with any reasonable expectation of success, to treat inflammation, atherosclerosis or hypertension as asserted by the Examiner.

Moreover, Applicants have discovered *unexpected benefits* of cocoa procyanidin monomers and/or oligomers on cardiovascular health and inflammation which were neither taught nor suggested by Lunder and Ho. First, Applicants have discovered that their compounds act as non-steroidal anti-inflammatory agents (*see e.g.* specification, page 18, under "Formulations and Methods"). This discovery is important because a replacement for aspirin, which has known side effects (stomach bleeding), is needed. This activity of cocoa procyanidin monomers and/or oligomers involves the arachidonic acid and eicosanoids pathway and could not have been expected based on anti-oxidant and lypoxygenase inhibiting effects of some tea polyphenols disclosed by Lunder and Ho. Additionally, Applicants have discovered that their compounds can *increase* nitric oxide (NO) synthesis (*see e.g.* specification, pages15-16). It si important that solely based on the antioxidant properties of some tea catechins, a person of skill in the art would have expected that NO, being a radical, would have been scavenged (*i.e.*, its amount reduced) by those compounds. In fact, persons of skill in the art believed that flavonoids were NO scavengers as an extension of their antioxidant activities (*see* Attachment III, van Acker *et al.*, Flavonoids as Scavengers of Nitric Oxide Radicals, *Biochem. Biophys. Res.*

Commn., 214: 755-759 (1995)). NO has a profound effect on atherosclerosis and NO-dependent hypertension. Finally, in addition to the effects that NO (and thus cocoa procyanidin monomers and/or oligomers) has on platelet aggregation, Applicants show that platelet activation may be prevented by suppressing activation of the GPIIbIIIa receptor for adhesive proteins.

Because cocoa procyanidin monomers and/or oligomers act on several independent mechanisms in the body of a human or a veterinary animal, a much more comprehensive effect on the health of the human or the veterinary animal can be achieved by administering these compounds pursuant to the therapeutic regiments of claims 186-224. Lunder and Ho fail to suggest such sweeping health effects.

Therefore, claims 186-192 (directed to treatment of NO-dependent hypertension), claims 205-215 (directed, *inter alia*, to treating atherosclerosis) and claims 216-224 (directed to treating inflammation) are not obvious over the cited prior art.

Claims 194-204 directed to anti-platelet therapy or prophylaxis are also rejected as obvious over Lunder in view of Ho. As noted above, Lunder discloses antioxidant effects, and Ho discloses antioxidant and soybean lipoxygenase effects of certain tea polyphenols. There is neither any teaching, nor any suggestion, in the cited prior art that anti-platelet effects could be achieved with any of the tea polyphenols, let alone the procyanidin monomers and/or oligomers recited in claims 194-204. As shown in the present specification, the anti-platelet application of the claimed compounds arises from their effect on cellular mechanisms that are independent from anti-oxidant and lypoxygenase inhibition effects. Withdrawal of the rejection of claims 194-204 is, therefore, respectfully requested.

In view of the above arguments, Applicants respectfully request withdrawal of the prior art rejection relating to method claims 186-224.

Obviousness-type Double Patenting Rejection

Claims 186-224 stand provisionally rejected as unpatentable over the claims of the co-pending applications Ser. Nos. 09/507,717, 09/717,833 and 09/717,893.

Applicants will file a Terminal Disclaimer to overcome the rejection over the claims of the U.S. Appl. Ser. No. 09/717,893 upon the indication of claim allowance in the present application.

With respect to the rejections over the '717 and the '833 applications, Applicants respectfully traverse.

The claims of the '717 application are directed to the therapeutic methods achieved by administering a *methylated* cocoa procyanidin, *i.e.,* a derivative of the cocoa procyanidin. The present claims are directed to the compounds that re *not* methylated and would not have been obvious over the compounds and methods recited in the claims of the '717 application. Therefore, withdrawal of the rejection is respectfully requested.

The claims of the '833 applications recite *in vitro* assays for identifying genes the expression of which is affected by cocoa procyanidins. The present claims in contrast recite method of treatment using such compounds, which are not obvious over the assay claims. Therefore, withdrawal of the rejection is respectfully requested.

Information Disclosure Statement filed on February 25, 2002

Applicants respectfully request that the Examiner considers the Information Disclosure Statement (IDS) filed on February 25, 2002. The Examiner has returned the form PTO-1449 with the instant Official Action but has not indicated that references were considered. In fact, the Examiner has crossed out all the references cited by the Applicants as "Duplicative," presumably in view of the references cited in the parent application U.S. Ser. No. 08/831,245.

Applicants thank the Examiner for considering the references filed in the parent '245 application with respect to presently pending claims, and apologize for any duplicate entries made on the February 25, 2002 IDS.

However, it appears to Applicants that, with the exception of several references, the publications listed were not duplicative. Applicants, therefore, respectfully request that the Examiner considers these publications and indicate that they were considered on the attached clean copy of the PTO-1449 originally filed on February 25, 2002 (On the PTO-1449, Applicants have crossed out the publications that they determined were considered by the Examiner as a result of the earlier filed IDSs.)

CONCLUSION

In view of the above amendments and remarks, Applicants believe that the application is now in condition for allowance. A notice to that effect is respectfully requested.

Date: June 21, 2002

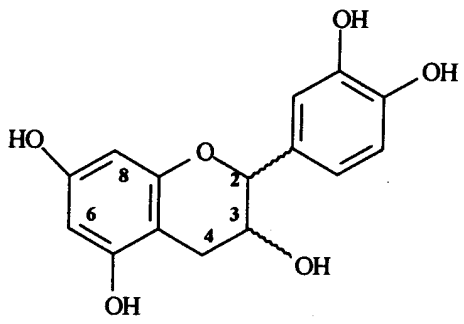
Respectfully submitted,

Nada Jain
Reg. No. 41,431

Clifford Chance Rogers & Wells LLP
200 Park Avenue
New York, NY 10166-0153
Telephone: (212) 878-8000

New Claims

120. A composition comprising a cocoa procyanidin monomer and/or oligomer and a carrier selected from the group consisting of a pharmaceutically acceptable carrier, veterinary acceptable carrier, dietary supplement carrier and food, wherein said composition is packaged with instructions directing use of the composition as an anti-inflammatory agent, and wherein the cocoa procyanidin monomer is of the formula:



and the cocoa procyanidin oligomer is composed of the monomers connected via interflavan linkages 4→6 and/or 4→8.

121. The composition of claim 120, wherein the cocoa procyanidin is a dimer.

122. The composition of claim 120, wherein the cocoa procyanidin is at least one of oligomers 3-12 or any mixture thereof.

123. The composition of claim 120, wherein the cocoa monomer and/or oligomer is in the form of a cocoa extract or cocoa procyanidin-containing fraction thereof.

124. The composition of claim 120, wherein the monomer comprises epicatechin and the oligomer comprises an epicatechin-containing oligomer.

125. The composition of claim 120, wherein the carrier is a pharmaceutically acceptable carrier.

126. The composition of claim 120, wherein the carrier is a veterinary acceptable carrier.

127. The composition of claim 120, wherein the carrier is a food.

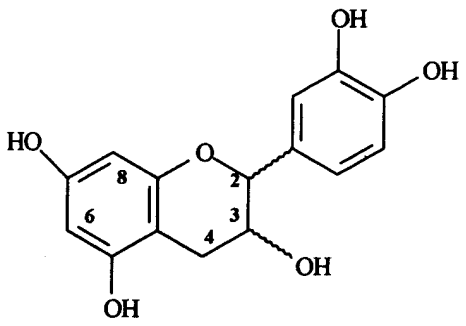
128. The composition of claim 120, which is a dietary supplement.

129. The packaged composition of claim 120, further comprising a cyclo-oxygenase modulator.

130. The packaged composition of claim 129, wherein the cyclo-oxygenase modulator is a non-steroidal anti-inflammatory drug.

131. The packaged composition of claim 130, wherein the non-steroidal anti-inflammatory drug is an aspirin.

132. A composition comprising a cocoa procyanidin monomer and/or oligomer and a carrier selected from the group consisting of a pharmaceutically acceptable carrier, veterinary acceptable carrier, dietary supplement carrier and food, wherein said composition is packaged with instructions directing use of the composition as an antiplatelet therapy, and wherein the cocoa procyanidin monomer is of the formula:



and the cocoa procyanidin oligomer is composed of the monomers connected via interflavan linkages 4→6 and/or 4→8.

133. The composition of claim 132, wherein the cocoa procyanidin is a dimer.

134. The composition of claim 132, wherein the cocoa procyanidin is at least one of oligomers 3-12 or any mixture thereof.

135. The composition of claim 132, wherein the cocoa monomer and/or oligomer is in the form of a cocoa extract or cocoa procyanidin-containing fraction thereof.

136. The composition of claim 132, wherein the monomer comprises epicatechin and the oligomer comprises an epicatechin-containing oligomer.

137. The composition of claim 132, , wherein the carrier is a pharmaceutically acceptable carrier.

138. The composition of claim 132, wherein the carrier is a veterinary acceptable carrier.

139. The composition of claim 132, wherein the carrier is a food.

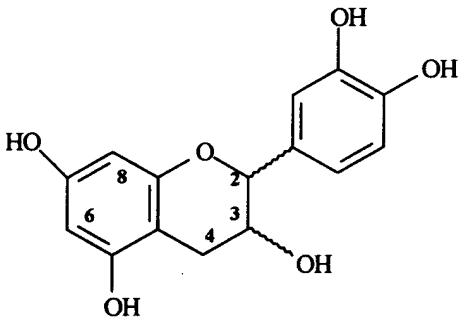
140. The composition of claim 132, which is a dietary supplement.

141. The packaged composition of claim 132, further comprising a cyclo-oxygenase modulator.

142. The packaged composition of claim 141, wherein the cyclo-oxygenase modulator is a non-steroidal anti-inflammatory drug.

143. The packaged composition of claim 142, wherein the non-steroidal anti-inflammatory drug is an aspirin.

144. A composition comprising a cocoa procyanidin monomer and/or oligomer and a carrier selected from the group consisting of a pharmaceutically acceptable carrier, veterinary acceptable carrier, dietary supplement carrier and food, wherein said composition is packaged with instructions directing use of the composition as an agent for improving or maintaining vascular health, and wherein the cocoa procyanidin monomer is of the formula:



and the cocoa procyanidin oligomer is composed of the monomers connected via interflavan linkages 4→6 and/or 4→8.

145. The composition of claim 144, wherein the cocoa procyanidin is a dimer.

146. The composition of claim 144, wherein the cocoa procyanidin is at least one of oligomers 3-12 or any mixture thereof.

147. The composition of claim 144, wherein the cocoa monomer and/or oligomer is in the form of a cocoa extract or cocoa procyanidin-containing fraction thereof.

148. The composition of claim 144, wherein the monomer comprises epicatechin and the oligomer comprises an epicatechin-containing oligomer.

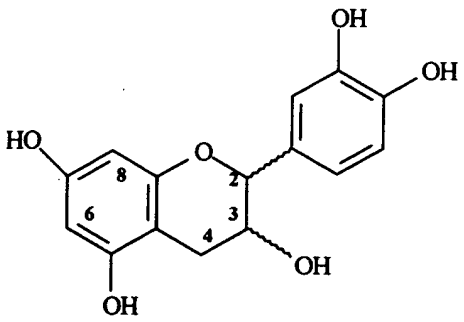
149. The composition of claim 144, wherein the carrier is a pharmaceutically acceptable carrier.

150. The composition of claim 144, wherein the carrier is a veterinary acceptable carrier.

151. The composition of claim 144, wherein the carrier is a food.

152. The composition of claim 144, which is a dietary supplement.

153. A composition comprising a cocoa procyanidin monomer and/or oligomer and a carrier selected from the group consisting of a pharmaceutically acceptable carrier, veterinary acceptable carrier, dietary supplement carrier and food, wherein said composition is packaged with instructions directing use of the composition for at least one of the following: modulating nitric oxide synthesis, inducing vasodilation, modulating renal function, and reducing blood pressure, and wherein the cocoa procyanidin monomer is of the formula:



and the cocoa procyanidin oligomer is composed of the monomers connected via interflavan linkages 4→6 and/or 4→8.

154. The composition of claim 153, wherein the cocoa procyanidin is a dimer.

155. The composition of claim 153, wherein the cocoa procyanidin is at least one of oligomers 3-12 or any mixture thereof.

156. The composition of claim 153, wherein the cocoa monomer and/or oligomer is in the form of a cocoa extract or cocoa procyanidin-containing fraction thereof.

157. The composition of claim 153, wherein the monomer comprises epicatechin and the oligomer comprises an epicatechin-containing oligomer.

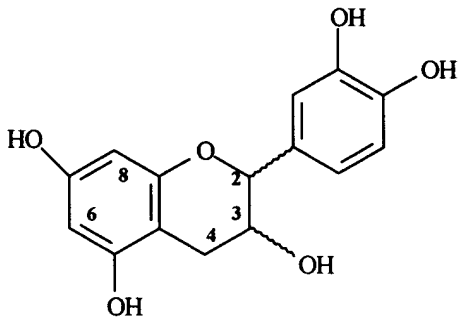
158. The composition of claim 153, wherein the carrier is a pharmaceutically acceptable carrier.

159. The composition of claim 153, wherein the carrier is a veterinary acceptable carrier.

160. The composition of claim 153, wherein the carrier is a food.

161. The composition of claim 153, which is a dietary supplement.

162. A composition comprising a cocoa procyanidin monomer and/or oligomer and a carrier selected from the group consisting of a pharmaceutically acceptable carrier, veterinary acceptable carrier, dietary supplement carrier and food, wherein said composition is packaged with instructions directing use of the composition for at least one of the following: reducing the risk of thrombosis, treating or preventing atherosclerosis, and treating or preventing restenosis, and wherein the cocoa procyanidin monomer is of the formula:



and the cocoa procyanidin oligomer is composed of the monomers connected via interflavan linkages 4→6 and/or 4→8.

163. The composition of claim 162, wherein the cocoa procyanidin is a dimer.

164. The composition of claim 162, wherein the cocoa procyanidin is at least one of oligomers 3-12 or any mixture thereof.

165. The composition of claim 162, wherein the cocoa monomer and/or oligomer is in the form of a cocoa extract or cocoa procyanidin-containing fraction thereof.

166. The composition of claim 162, wherein the monomer comprises epicatechin and the oligomer comprises an epicatechin-containing oligomer.

167. The composition of claim 162, wherein the carrier is a pharmaceutically acceptable carrier.

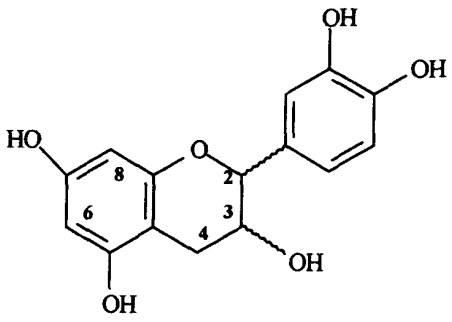
168. The composition of claim 162, wherein the carrier is a veterinary acceptable carrier.

169. The composition of claim 162, wherein the carrier is a food.

170. The composition of claim 162, which is a dietary supplement.

171. A composition comprising a cocoa procyanidin monomer and/or oligomer and a carrier selected from the group consisting of a pharmaceutically acceptable carrier, veterinary

acceptable carrier, dietary supplement carrier and food, wherein said composition is packaged with instructions directing use of the composition for treating NO-dependent hypertension, and wherein the cocoa procyanidin monomer is of the formula:



and the cocoa procyanidin oligomer is composed of the monomers connected via interflavan linkages 4→6 and/or 4→8.

172. The composition of claim 171, wherein the cocoa procyanidin is a dimer.

173. The composition of claim 171, wherein the cocoa procyanidin is at least one of oligomers 3-12 or any mixture thereof.

174. The composition of claim 171, wherein the cocoa monomer and/or oligomer is in the form of a cocoa extract or cocoa procyanidin-containing fraction thereof.

175. The composition of claim 171, wherein the monomer comprises epicatechin and the oligomer comprises an epicatechin-containing oligomer.

176. The composition of claim 171, wherein the carrier is a pharmaceutically acceptable carrier.

177. The composition of claim 171, wherein the carrier is a veterinary acceptable carrier.

178. The composition of claim 171, wherein the carrier is a food.

179. The composition of claim 171, which is a dietary supplement.

180. A composition comprising an effective amount of cocoa procyanidin monomer and/or oligomer in admixture with a cyclo-oxygenase modulator.

181. The composition of claim 180, wherein the cyclo-oxygenase modulator is a non-steroidal anti-inflammatory drug.

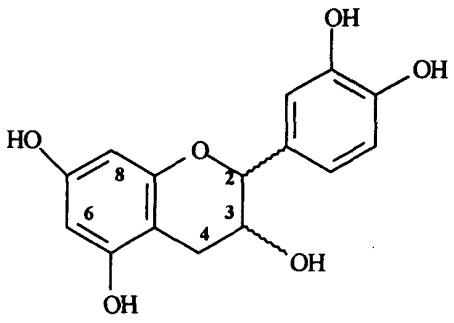
182. The composition of claim 181, wherein the non-steroidal anti-inflammatory drug is an aspirin.

183. The composition of claim 180, wherein the cocoa procyanidin is a dimer.

184. The composition of claim 180, wherein the cocoa monomer and/or oligomer is in the form of a cocoa extract or cocoa procyanidin-containing fraction thereof.

185. The composition of claim 180, wherein the monomer comprises epicatechin and the oligomer comprises an epicatechin-containing oligomer.

186. A method of treating NO-dependent hypertension by administering a composition comprising a cocoa procyanidin monomer and/or oligomer and a carrier selected from the group consisting of a pharmaceutically acceptable carrier, veterinary acceptable carrier, dietary supplement carrier and food to a subject suffering from NO-dependent hypertension, wherein said subject is a human or a veterinary animal, and wherein the cocoa procyanidin monomer is of the formula:



and the cocoa procyanidin oligomer is composed of the monomers connected via interflavan linkages 4→6 and/or 4→8.

187. The method of claim 186, wherein said subject is a human.

188. The method of claim 186, wherein the cocoa procyanidin is a dimer.

189. The composition of claim 186, wherein the cocoa monomer and/or oligomer is in the form of a cocoa extract or cocoa procyanidin-containing fraction thereof.

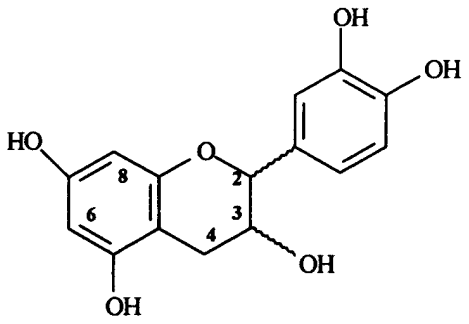
190. The composition of claim 186, wherein the monomer comprises epicatechin and the oligomer comprises an epicatechin-containing oligomer.

191. The method of claim 186, wherein the cocoa procyanidin is at least one of oligomers 3-12 or any mixture thereof.

192. The method of claim 191, wherein the carrier is a pharmaceutically acceptable carrier.

193. The method of claim 186 wherein the carrier is a food.

194. A method of anti-platelet therapy or prophylaxis comprising administering to a subject in need thereof a composition comprising an effective amount of a cocoa procyanidin monomer and/or oligomer and a carrier selected from the group consisting of a pharmaceutically acceptable carrier, veterinary acceptable carrier, dietary supplement carrier and food, wherein said subject is a human or a veterinary animal, and wherein the cocoa procyanidin monomer is of the formula:



and the cocoa procyanidin oligomer is composed of the monomers connected via interflavan linkages 4→6 and/or 4→8.

195. The method of claim 194, wherein said subject is a human.

196. The method of claim 194, wherein the cocoa procyanidin is a dimer.

197. The composition of claim 194, wherein the cocoa monomer and/or oligomer is in the form of a cocoa extract or cocoa procyanidin-containing fraction thereof.

198. The composition of claim 194, wherein the monomer comprises epicatechin and the oligomer comprises an epicatechin-containing oligomer.

199. The method of claim 194, wherein the cocoa procyanidin is at least one of oligomers 3-12 or any mixture thereof.

200. The method of claim 199, wherein the carrier is a pharmaceutically acceptable carrier.

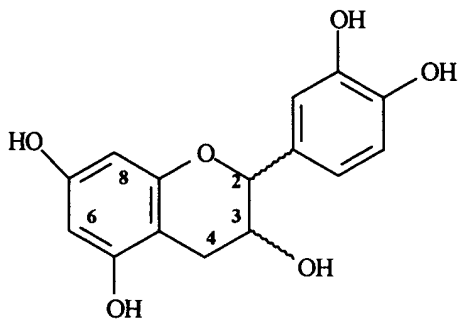
201. The method of claim 194 wherein the carrier is a food.

202. The method of claim 194 further comprising administering to the subject a cyclo-oxygenase modulator.

203. The method of claim 202, wherein the cyclo-oxygenase modulator is a non-steroidal anti-inflammatory drug.

204. The method of claim 203, wherein the non-steroidal anti-inflammatory drug is an aspirin.

205. A method of treating, reducing the risk of, or preventing atherosclerosis, thrombosis, restenosis, heart attack or stroke comprising administering to a subject in need thereof a composition comprising an effective amount of a cocoa procyanidin monomer and/or oligomer and a carrier selected from the group consisting of a pharmaceutically acceptable carrier, veterinary acceptable carrier, dietary supplement carrier and food, wherein said subject is a human or a veterinary animal, and wherein the cocoa procyanidin monomer is of the formula:



and the cocoa procyanidin oligomer is composed of the monomers connected via interflavan linkages 4→6 and/or 4→8.

206. The method of claim 205, wherein said subject is a human.

207. The method of claim 205, wherein the cocoa procyanidin is a dimer.

208. The composition of claim 205, wherein the cocoa monomer and/or oligomer is in the form of a cocoa extract or cocoa procyanidin-containing fraction thereof.

209. The composition of claim 205, wherein the monomer comprises epicatechin and the oligomer comprises an epicatechin-containing oligomer.

210. The method of claim 209, wherein the cocoa procyanidin is at least one of oligomers 3-12 or any mixture thereof.

211. The method of claim 210, wherein the carrier is a pharmaceutically acceptable carrier.

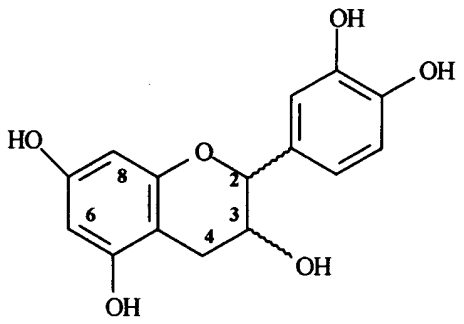
212. The method of claim 205, wherein the carrier is a food.

213. The method of claim 205, further comprising administering to the subject a cyclo-oxygenase modulator.

214. The method of claim 213, wherein the cyclo-oxygenase modulator is a non-steroidal anti-inflammatory drug.

215. The method of claim 214, wherein the non-steroidal anti-inflammatory drug is an aspirin.

216. A method of treating or reducing the progression of a condition associated with inflammation comprising administering to a subject in need thereof a composition comprising an effective amount of a cocoa procyanidin monomer and/or oligomer and a carrier selected from the group consisting of a pharmaceutically acceptable carrier, veterinary acceptable carrier, dietary supplement carrier and food, wherein said subject is a human or a veterinary animal, and wherein the cocoa procyanidin monomer is of the formula:



and the cocoa procyanidin oligomer is composed of the monomers connected via interflavan linkages 4→6 and/or 4→8.

217. The method of claim 216, wherein said subject is a human.

218. The method of claim 217, wherein the condition associated with inflammation is at least one of the following: inflammatory bowel disease, ulcerative colitis, Chron's disease, gingivitis, acute edema, chronic arthritis, and spondylitis.

219. The method of claim 216, wherein the cocoa procyanidin is a dimer.

220. The composition of claim 216, wherein the cocoa monomer and/or oligomer is in the form of a cocoa extract or cocoa procyanidin-containing fraction thereof.

221. The composition of claim 216, wherein the monomer comprises epicatechin and the oligomer comprises an epicatechin-containing oligomer.

222. The method of claim 216, wherein the cocoa procyanidin is at least one of oligomers 3-12 or any mixture thereof.

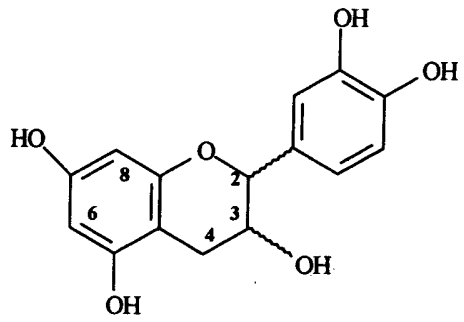
223. The method of claim 222, wherein the carrier is a pharmaceutically acceptable carrier.

224. The method of claim 216 wherein the carrier is a food.--

ATTACHMENT I

Claims Amendments

120. A composition comprising a cocoa procyanidin monomer and/or oligomer and a carrier selected from the group consisting of a pharmaceutically acceptable carrier, veterinary acceptable carrier, dietary supplement carrier and food, wherein said composition is packaged with instructions directing use of the composition as an anti-inflammatory agent, and wherein the cocoa procyanidin monomer is of the formula:



and the cocoa procyanidin oligomer is composed of the monomers connected via interflavan linkages 4→6 and/or 4→8.

121. The composition of claim 120, wherein the cocoa procyanidin is a dimer.

122. The composition of claim 120, wherein the cocoa procyanidin is at least one of oligomers 3-12 or any mixture thereof.

123. The composition of claim 120, wherein the cocoa monomer and/or oligomer is in the form of a cocoa extract or cocoa procyanidin-containing fraction thereof.

124. The composition of claim 120, wherein the monomer comprises epicatechin and the oligomer comprises an epicatechin-containing oligomer.

125. The composition of claim 120, wherein the carrier is a pharmaceutically acceptable carrier.

126. The composition of claim 120, wherein the carrier is a veterinary acceptable carrier.

127. The composition of claim 120, wherein the carrier is a food.

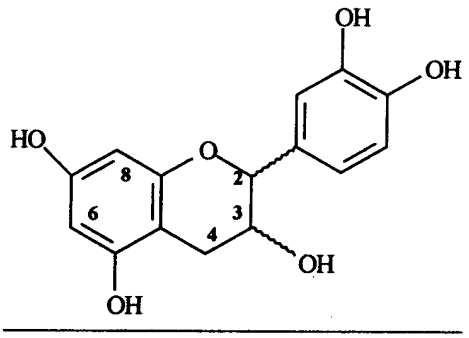
128. The composition of claim 120, which is a dietary supplement.

129. The packaged composition of claim 120, further comprising a cyclo-oxygenase modulator.

130. The packaged composition of claim 129, wherein the cyclo-oxygenase modulator is a non-steroidal anti-inflammatory drug.

131. The packaged composition of claim 130, wherein the non-steroidal anti-inflammatory drug is an aspirin.

132. A composition comprising a cocoa procyanidin monomer and/or oligomer and a carrier selected from the group consisting of a pharmaceutically acceptable carrier, veterinary acceptable carrier, dietary supplement carrier and food, wherein said composition is packaged with instructions directing use of the composition as an antiplatelet therapy, and wherein the cocoa procyanidin monomer is of the formula:



and the cocoa procyanidin oligomer is composed of the monomers connected via interflavan linkages 4→6 and/or 4→8.

133. The composition of claim 132, wherein the cocoa procyanidin is a dimer.

134. The composition of claim 132, wherein the cocoa procyanidin is at least one of oligomers 3-12 or any mixture thereof.

135. The composition of claim 132, wherein the cocoa monomer and/or oligomer is in the form of a cocoa extract or cocoa procyanidin-containing fraction thereof.

136. The composition of claim 132, wherein the monomer comprises epicatechin and the oligomer comprises an epicatechin-containing oligomer.

137. The composition of claim 132, , wherein the carrier is a pharmaceutically acceptable carrier.

138. The composition of claim 132, wherein the carrier is a veterinary acceptable carrier.

139. The composition of claim 132, wherein the carrier is a food.

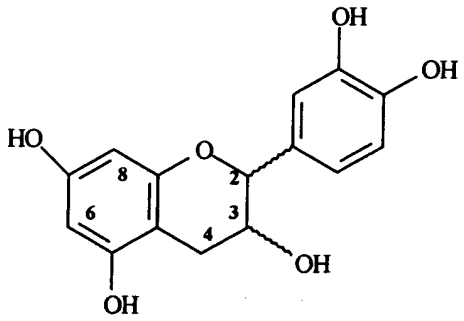
140. The composition of claim 132, which is a dietary supplement.

141. The packaged composition of claim 132, further comprising a cyclo-oxygenase modulator.

142. The packaged composition of claim 141, wherein the cyclo-oxygenase modulator is a non-steroidal anti-inflammatory drug.

143. The packaged composition of claim 142, wherein the non-steroidal anti-inflammatory drug is an aspirin.

144. A composition comprising a cocoa procyanidin monomer and/or oligomer and a carrier selected from the group consisting of a pharmaceutically acceptable carrier, veterinary acceptable carrier, dietary supplement carrier and food, wherein said composition is packaged with instructions directing use of the composition as an agent for improving or maintaining vascular health, and wherein the cocoa procyanidin monomer is of the formula:



and the cocoa procyanidin oligomer is composed of the monomers connected via interflavan linkages 4→6 and/or 4→8.

145. The composition of claim 144, wherein the cocoa procyanidin is a dimer.

146. The composition of claim 144, wherein the cocoa procyanidin is at least one of oligomers 3-12 or any mixture thereof.

147. The composition of claim 144, wherein the cocoa monomer and/or oligomer is in the form of a cocoa extract or cocoa procyanidin-containing fraction thereof.

148. The composition of claim 144, wherein the monomer comprises epicatechin and the oligomer comprises an epicatechin-containing oligomer.

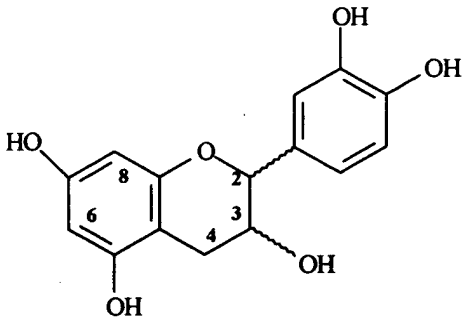
149. The composition of claim 144, wherein the carrier is a pharmaceutically acceptable carrier.

150. The composition of claim 144, wherein the carrier is a veterinary acceptable carrier.

151. The composition of claim 144, wherein the carrier is a food.

152. The composition of claim 144, which is a dietary supplement.

153. A composition comprising a cocoa procyanidin monomer and/or oligomer and a carrier selected from the group consisting of a pharmaceutically acceptable carrier, veterinary acceptable carrier, dietary supplement carrier and food, wherein said composition is packaged with instructions directing use of the composition for at least one of the following: modulating nitric oxide synthesis, inducing vasodilation, modulating renal function, and reducing blood pressure, and wherein the cocoa procyanidin monomer is of the formula:



and the cocoa procyanidin oligomer is composed of the monomers connected via interflavan linkages 4→6 and/or 4→8.

154. The composition of claim 153, wherein the cocoa procyanidin is a dimer.

155. The composition of claim 153, wherein the cocoa procyanidin is at least one of oligomers 3-12 or any mixture thereof.

156. The composition of claim 153, wherein the cocoa monomer and/or oligomer is in the form of a cocoa extract or cocoa procyanidin-containing fraction thereof.

157. The composition of claim 153, wherein the monomer comprises epicatechin and the oligomer comprises an epicatechin-containing oligomer.

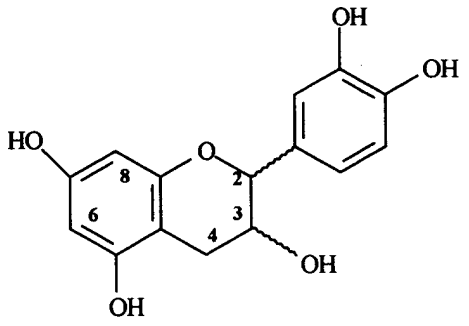
158. The composition of claim 153, wherein the carrier is a pharmaceutically acceptable carrier.

159. The composition of claim 153, wherein the carrier is a veterinary acceptable carrier.

160. The composition of claim 153, wherein the carrier is a food.

161. The composition of claim 153, which is a dietary supplement.

162. A composition comprising a cocoa procyanidin monomer and/or oligomer and a carrier selected from the group consisting of a pharmaceutically acceptable carrier, veterinary acceptable carrier, dietary supplement carrier and food, wherein said composition is packaged with instructions directing use of the composition for at least one of the following: reducing the risk of thrombosis, treating or preventing atherosclerosis, and treating or preventing restenosis, and wherein the cocoa procyanidin monomer is of the formula:



and the cocoa procyanidin oligomer is composed of the monomers connected via interflavan linkages 4→6 and/or 4→8.

163. The composition of claim 162, wherein the cocoa procyanidin is a dimer.

164. The composition of claim 162, wherein the cocoa procyanidin is at least one of oligomers 3-12 or any mixture thereof.

165. The composition of claim 162, wherein the cocoa monomer and/or oligomer is in the form of a cocoa extract or cocoa procyanidin-containing fraction thereof.

166. The composition of claim 162, wherein the monomer comprises epicatechin and the oligomer comprises an epicatechin-containing oligomer.

167. The composition of claim 162, wherein the carrier is a pharmaceutically acceptable carrier.

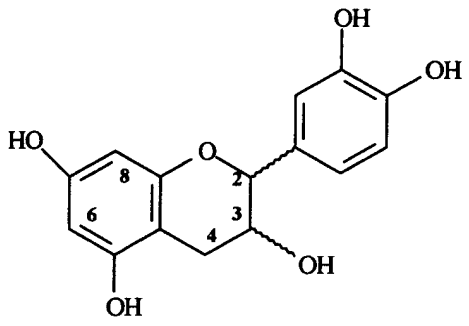
168. The composition of claim 162, wherein the carrier is a veterinary acceptable carrier.

169. The composition of claim 162, wherein the carrier is a food.

170. The composition of claim 162, which is a dietary supplement.

171. A composition comprising a cocoa procyanidin monomer and/or oligomer and a carrier selected from the group consisting of a pharmaceutically acceptable carrier, veterinary

acceptable carrier, dietary supplement carrier and food, wherein said composition is packaged with instructions directing use of the composition for treating NO-dependent hypertension, and wherein the cocoa procyanidin monomer is of the formula:



and the cocoa procyanidin oligomer is composed of the monomers connected via interflavan linkages 4→6 and/or 4→8.

172. The composition of claim 171, wherein the cocoa procyanidin is a dimer.

173. The composition of claim 171, wherein the cocoa procyanidin is at least one of oligomers 3-12 or any mixture thereof.

174. The composition of claim 171, wherein the cocoa monomer and/or oligomer is in the form of a cocoa extract or cocoa procyanidin-containing fraction thereof.

175. The composition of claim 171, wherein the monomer comprises epicatechin and the oligomer comprises an epicatechin-containing oligomer.

176. The composition of claim 171, wherein the carrier is a pharmaceutically acceptable carrier.

177. The composition of claim 171, wherein the carrier is a veterinary acceptable carrier.

178. The composition of claim 171, wherein the carrier is a food.

179. The composition of claim 171, which is a dietary supplement.

180. A composition comprising an effective amount of cocoa procyanidin monomer and/or oligomer in admixture with a cyclo-oxygenase modulator.

181. The composition of claim 180, wherein the cyclo-oxygenase modulator is a non-steroidal anti-inflammatory drug.

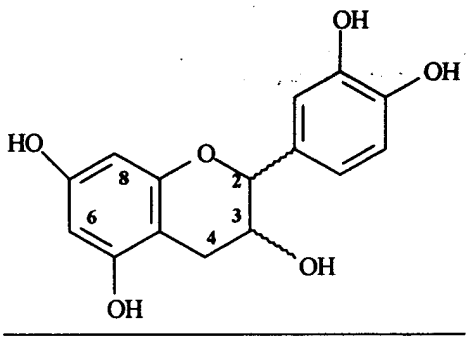
182. The composition of claim 181, wherein the non-steroidal anti-inflammatory drug is an aspirin.

183. The composition of claim 180, wherein the cocoa procyanidin is a dimer.

184. The composition of claim 180, wherein the cocoa monomer and/or oligomer is in the form of a cocoa extract or cocoa procyanidin-containing fraction thereof.

185. The composition of claim 180, wherein the monomer comprises epicatechin and the oligomer comprises an epicatechin-containing oligomer.

186. A method of treating NO-dependent hypertension by administering a composition comprising a cocoa procyanidin monomer and/or oligomer and a carrier selected from the group consisting of a pharmaceutically acceptable carrier, veterinary acceptable carrier, dietary supplement carrier and food to a subject suffering from NO-dependent hypertension, wherein said subject is a human or a veterinary animal, and wherein the cocoa procyanidin monomer is of the formula:



and the cocoa procyanidin oligomer is composed of the monomers connected via interflavan linkages 4→6 and/or 4→8.

187. The method of claim 186, wherein said subject is a human.

188. The method of claim 186, wherein the cocoa procyanidin is a dimer.

189. The composition of claim 186, wherein the cocoa monomer and/or oligomer is in the form of a cocoa extract or cocoa procyanidin-containing fraction thereof.

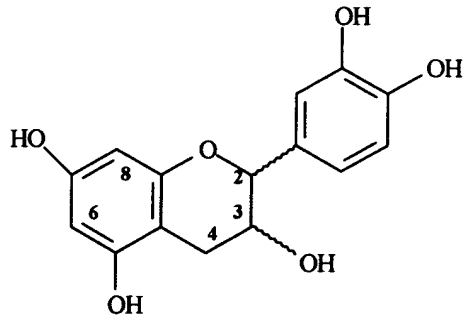
190. The composition of claim 186, wherein the monomer comprises epicatechin and the oligomer comprises an epicatechin-containing oligomer.

191. The method of claim 186, wherein the cocoa procyanidin is at least one of oligomers 3-12 or any mixture thereof.

192. The method of claim 191, wherein the carrier is a pharmaceutically acceptable carrier.

193. The method of claim 186 wherein the carrier is a food.

194. A method of anti-platelet therapy or prophylaxis comprising administering to a subject in need thereof a composition comprising an effective amount of a cocoa procyanidin monomer and/or oligomer and a carrier selected from the group consisting of a pharmaceutically acceptable carrier, veterinary acceptable carrier, dietary supplement carrier and food, wherein said subject is a human or a veterinary animal, and wherein the cocoa procyanidin monomer is of the formula:



and the cocoa procyanidin oligomer is composed of the monomers connected via interflavan linkages 4→6 and/or 4→8.

195. The method of claim 194, wherein said subject is a human.

196. The method of claim 194, wherein the cocoa procyanidin is a dimer.

197. The composition of claim 194, wherein the cocoa monomer and/or oligomer is in the form of a cocoa extract or cocoa procyanidin-containing fraction thereof.

198. The composition of claim 194, wherein the monomer comprises epicatechin and the oligomer comprises an epicatechin-containing oligomer.

199. The method of claim 194, wherein the cocoa procyanidin is at least one of oligomers 3-12 or any mixture thereof.

200. The method of claim 199, wherein the carrier is a pharmaceutically acceptable carrier.

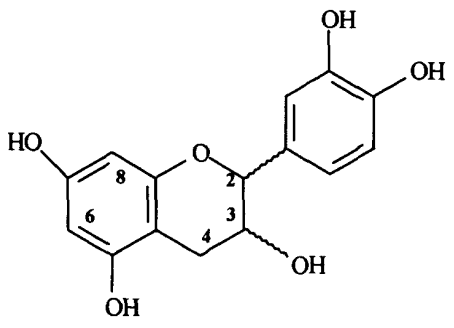
201. The method of claim 194 wherein the carrier is a food.

202. The method of claim 194 further comprising administering to the subject a cyclo-oxygenase modulator.

203. The method of claim 202, wherein the cyclo-oxygenase modulator is a non-steroidal anti-inflammatory drug.

204. The method of claim 203, wherein the non-steroidal anti-inflammatory drug is an aspirin.

205. A method of treating, reducing the risk of, or preventing atherosclerosis, thrombosis, restenosis, heart attack or stroke comprising administering to a subject in need thereof a composition comprising an effective amount of a cocoa procyanidin monomer and/or oligomer and a carrier selected from the group consisting of a pharmaceutically acceptable carrier, veterinary acceptable carrier, dietary supplement carrier and food, wherein said subject is a human or a veterinary animal, and wherein the cocoa procyanidin monomer is of the formula:



and the cocoa procyanidin oligomer is composed of the monomers connected via interflavan linkages 4→6 and/or 4→8.

206. The method of claim 205, wherein said subject is a human.

207. The method of claim 205, wherein the cocoa procyanidin is a dimer.

208. The composition of claim 205, wherein the cocoa monomer and/or oligomer is in the form of a cocoa extract or cocoa procyanidin-containing fraction thereof.

209. The composition of claim 205, wherein the monomer comprises epicatechin and the oligomer comprises an epicatechin-containing oligomer.

210. The method of claim 209, wherein the cocoa procyanidin is at least one of oligomers 3-12 or any mixture thereof.

211. The method of claim 210, wherein the carrier is a pharmaceutically acceptable carrier.

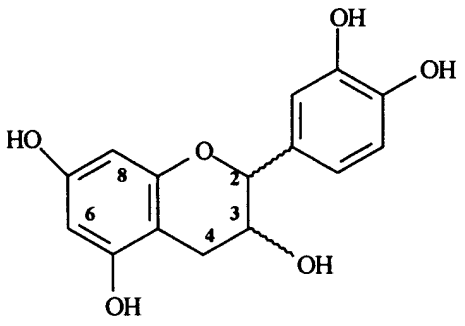
212. The method of claim 205, wherein the carrier is a food.

213. The method of claim 205, further comprising administering to the subject a cyclo-oxygenase modulator.

214. The method of claim 213, wherein the cyclo-oxygenase modulator is a non-steroidal anti-inflammatory drug.

215. The method of claim 214, wherein the non-steroidal anti-inflammatory drug is an aspirin.

216. A method of treating or reducing the progression of a condition associated with inflammation comprising administering to a subject in need thereof a composition comprising an effective amount of a cocoa procyanidin monomer and/or oligomer and a carrier selected from the group consisting of a pharmaceutically acceptable carrier, veterinary acceptable carrier, dietary supplement carrier and food, wherein said subject is a human or a veterinary animal, and wherein the cocoa procyanidin monomer is of the formula:



and the cocoa procyanidin oligomer is composed of the monomers connected via interflavan linkages 4→6 and/or 4→8.

217. The method of claim 216, wherein said subject is a human.

218. The method of claim 217, wherein the condition associated with inflammation is at least one of the following: inflammatory bowel disease, ulcerative colitis, Chron's disease, gingivitis, acute edema, chronic arthritis, and spondylitis.

219. The method of claim 216, wherein the cocoa procyanidin is a dimer.

220. The composition of claim 216, wherein the cocoa monomer and/or oligomer is in the form of a cocoa extract or cocoa procyanidin-containing fraction thereof.

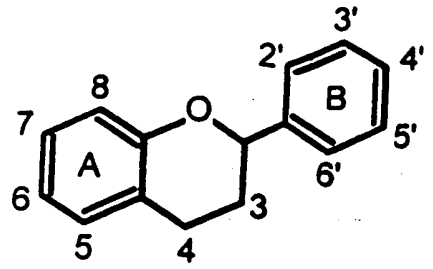
221. The composition of claim 216, wherein the monomer comprises epicatechin and the oligomer comprises an epicatechin-containing oligomer.

222. The method of claim 216, wherein the cocoa procyanidin is at least one of oligomers 3-12 or any mixture thereof.

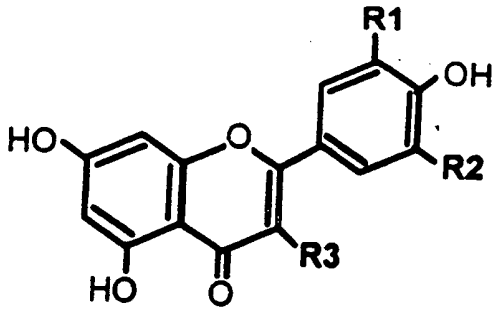
223. The method of claim 222, wherein the carrier is a pharmaceutically acceptable carrier.

224. The method of claim 216 wherein the carrier is a food.--

ATTACHMENT II

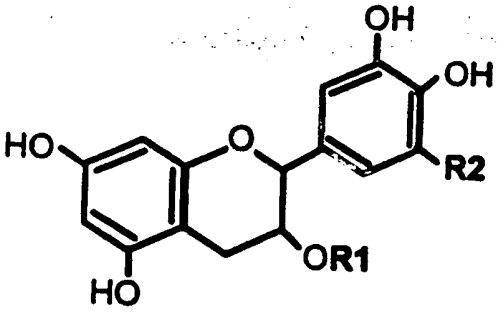


Flavonoid basic structure



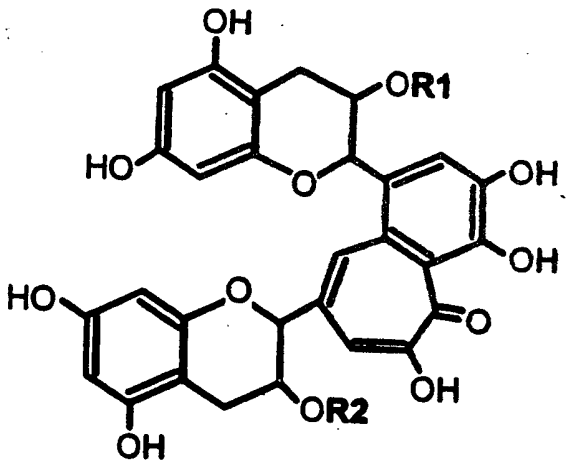
Flavon-3-ols

Quercetin	R1=OH, R2=H, R3= OH
Rutin	R1=OH, R2=H, R3=O-glycoside
Kaempferol	R1=H, R2=H, R3-OH



Flavan-3-ols (Catechins)

EC, C	: R, R2 = H
EGC	: R1 = H, R2 = OH
ECG	: R1 = gallate, R2 = H
EGCG	: R1 = gallate, R2 = OH



Theaflavins

Theaflavin:	R1, R2=H
Theaflavin-monogallate:	R1 or R2=gallate
Theaflavin-digallate:	R1,R2=gallate

FIGURE 1. Structures of catechins, flavonols, and theaflavins. Thearubigins are complex polymers of catechins with unknown structure.

ATTACHMENT III

Vol. 214, No. 3, 1995
September 25, 1995
BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pag s 755-759

FLAVONOIDS AS SCAVENGERS OF NITRIC OXIDE RADICAL

Sask[illegible] A.B.E. van Acker*#[1], Michèl N.J.L. Tromp*, Guido R.M.M. Haenen*, Wim J.F. van der Vijgh# and Aalt Bast*

*LACDR, Division of Molecular Pharmacology, Department of Pharmacochemistry, Faculty of Chemistry, Vrije Universiteit, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands

#Department of Medical Oncology, University Hospital Vrije Universiteit, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands

Received August 7, 1995

Flavonoids are a group of naturally occurring compounds used ,e.g., in the treatment of vascular endothelial damage. They are known to be excellent scavengers of oxygen free radicals. Since the nitric oxide radical ('NO) probably plays a role in this pathology, the 'NO scavenging capacity of the flavonoids was determined. It was found that the flavonoids are very potent 'NO scavengers. The anthocyanidins were found to be more effective scavengers than the hydroxyethylrutosides, which correlated with their therapeutic activity. The values of their scavenging rate constants are only 30 times less active than the very potent endogenous 'NO scavenger haemoglobin. It is speculated that 'NO scavenging plays a role in the therapeutic effect of the flavonoids.

Flavonoids are a group of naturally occurring antioxidants, usually found in plants, fruits and vegetables. As pure compounds or as mixtures, they are clinically used in diseases of the vascular wall involving inflammation and endothelial damage[1-4]. Venoruton, a semi-synthetic hydroxyethylrutoside mixture is used to reduce capillary permeability in chronic venous insufficiency and to protect the endothelial layer of blood vessels in diabetes mellitus[4].

In inflammation and endothelial damage free radicals play a major role. Of most radicals, both beneficial as well as undesirable effects are described [5]. This holds, especially, true for 'NO. On the one hand 'NO is a key mediator in various physiological processes.

[1]Corresponding author. Fax:+31204447610.

0006-291X/95 $12.00

On the other hand ·NO is toxic e.g. as precursor of peroxynitrite [6].

Part of the therapeutic effect of flavonoids has been ascribed to their free radical scavenging capacity. Because of the important role of ·NO, the scavenging of ·NO by therapeutically used flavonoids is studied.

Materials and Methods

Chemicals

Rutin was obtained from Merck and pelargonidin chloride was purchased from Fluka. Cyanidin chloride was purchased from Roth. The hydroxyethylrutosides and trihydroxyethyl quercetin were a generous gift from Zyma (Nyon, Switzerland). NO gas was from Messner Griesman, 99.95% pure.

NO scavenging

Deoxygenated water was purged with ·NO gas for about 1 min. Four µl of the ·NO saturated water was added to 20 ml 50 mM phosphate buffer (pH 7.4) in a thermostatted waterjacketed bath (37°C). The buffer was saturated with N_2 gas and during measurement the test vessel was kept under an N_2 atmosphere. The ·NO concentration was monitored with an iso-NO meter (World Precision Instruments Inc., Sarasota FL, USA) which was coupled to a MacLab interface (ML020 MacLab/8, ADInstruments Ltd, London, England) and an Apple Macintosh computer with 'Chart' software. The decrease in ·NO concentration was followed in time in the presence or absence of flavonoid. The flavonoids, in a stock concentration of 10 mM, were dissolved either in millipore water (tetraHER) or in 100 % DMSO (all other flavonoids). The final DMSO concentration was 1%, which has no effect on the measurements. The final concentration of flavonoid was 100 µM, or lower.

The log K values were calculated by plotting the ln ·NO concentration versus time, which resulted in a straight line. The reaction shows pseudo first order kinetics, as the scavenger is in excess. By dividing the pseudo first order rate constant (ks) by the concentration of scavenger, the scavenging rate constant (K) is obtained:

$$v = -\frac{d[NO]}{dt} = K[S][\cdot NO]$$

$$ks = K[S]$$

Corrections were made for the spontaneous degradation of ·NO (first order rate constant $1.0 \pm 0.1 \times 10^{-3}\ M^{-1}\ s^{-1}$).

Results and Discussion

It is known that some flavonoids are excellent scavengers of free radicals like the hydroxyl radical and superoxide anion radicals [7-9]. In the present study, we found that flavonoids are also excellent ·NO scavengers (table 1). Their activity exceeds that of glutathione 10 to 1000 times. For comparison, we determined the log K of haemoglobin, which was found to be 4.25. This means a K value of $1.78 \times 10^4\ M^{-1}\ s^{-1}$, which is

Table 1. log K values of the flavonoids tested

Flavonoid	log K ± SD ($M^{-1}s^{-1}$)	number of exp.
cyanidin	2.54 ± 0.24	3
pelargonidin	2.60 ± 0.08	3
monoHER	0.95 ± 0.05	4
diHER	0.87 ± 0.05	4
triHER	1.04 ± 0.03	4
tetraHER	0.99 ± 0.03	4
rutin	0.96 ± 0.06	4
haemoglobin	4.25 ± 0.23	2

comparable to the value of 2.1 x 10^4 M^{-1} s^{-1} reported by Alayash et al.[10]. The anthocyanidins, cyanidin and pelargonidin, appear to be more potent scavengers of NO compared to the hydroxyethyl rutosides. The halflife of ·NO is decreased by 50 % by adding only 5μM of pelargonidin, the most potent flavonoid studied (Fig. 1). The ·NO scavenging potency correlates with the therapeutic efficacy since the anthocyanidins are known to be more effective than the hydroxyethylrutosides in reducing capillary permeability and fragility and in their anti-inflammatory and anti-oedemic activities [11].

As mentioned above, ·NO has both beneficial as well as undesirable effects. ·NO is a mediator of inflammation [12, 13] and plays an essential role in the defence mechanism of macrophages against micro-organisms [14]. ·NO generated by inflammatory cells is toxic, probably after reaction with superoxide anion, which gives rise to peroxynitrite. Once protonated, peroxynitrite decays rapidly to form the reactive HO· and the stable NO_2· radical, as suggested by Beckman et al. [15]. Scavenging of these ·NO radicals will contribute to the therapeutic effect of the flavonoids.

In blood vessels ·NO is produced by endothelial cells, e.g. upon stimulation of muscarinergic receptors. The ·NO diffuses to the vascular smooth muscle cells where ·NO-induced cGMP formation leads to muscle relaxation. Scavenging of this ·NO will lead to vasoconstriction and, possibly, to vascular damage.

Flavonoids appear to accumulate between the endothelial layer and the vascular smooth muscle cells, where a high local concentration is reached [16]. This would favour the scavenging of the endothelial derived NO that is responsible for vasodilatation. However,

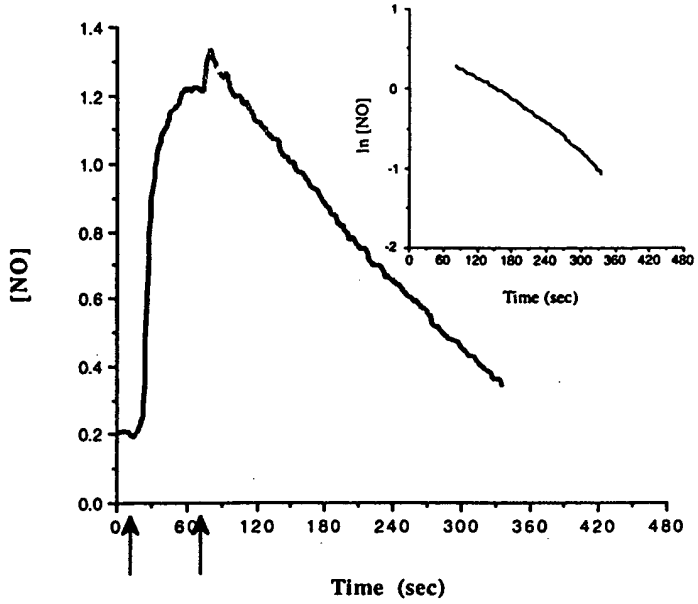


Fig. 1. A representative experiment with 5 µM Pelargonidine. At the timepoint of the first arrow 4 µl of NO saturated water is added to the testvessel. At the timepoint of the second arrow 200 µl of a 0.5 mM solution of Pelargonidine is added. The inset represents the ln [NO] vs time curve.

at the site where the flavonoids accumulate also arteriosclerosis begins in a process where undoubtedly 'NO radicals display an unfavourable role. A correlation has been found between the intake of flavonoids and a low incidence of cardiovascular diseases (the Zutphen Elderly study [17]). Therefore, it is tempting to speculate that 'NO scavenging of flavonoids plays an important role in their therapeutic effect.

If indeed 'NO scavenging is one of the major protective features of the flavonoids, we wonder how the scavenger is able to discriminate between the wanted and the deleterious 'NO. Probably the local distribution of the flavonoid has to be taken into consideration.

In conclusion, we demonstrate the potent 'NO scavenging effect of some flavonoids. It is speculated that this feature of the flavonoids is of importance in the therapeutic effect of these naturally occurring antioxidants.

References

1. (1994) Age 17, 1-6.
2. Brandi, M.L. (1992) Bone and Mineral 19, S3-S14.

3. Pathak, D., Pathak, K. and Singla, A.K. (1991) Fitoterapia LXII, 371-389.
4. Wadworth, A.N. and Faulds, D. (1992) Drugs 44, 1013-1032.
5. Bast, A., Haenen, G.R.M.M. and Doelman, C.J.A. (1991) Am. J. Med. 91, 3C-2S - 3C-13S.
6. Liew, F.Y. and Cox, F.E.G. (1991) Immun. Today March, A17-A21.
7. Husain, S.R., Cillard, J. and Cillard, P. (1987) Phytochemistry 26, 2489-2491.
8. Bors, W. and Saran, M. (1987) Free Rad. Res. Comm. 2, 289-294.
9. Jovanovic, S.V., Steenken, S., Tosic, M., Marjanovic, B. and Simic, M.G. (1994) J. Am. Chem. Soc. 116, 4846-4851.
10. Alayash, A.I., Frantantoni, J.C., Bonaventura, C., Bonaventura, J. and Cashon, R.E. (1993) Arch. Biochem. Biophys. 303, 332-338.
11. Wagner, H. (1985) in Annual proceedings of the phytochemical society of europe (C.F. Van Sumere and P.J. Lea, eds.), Vol. 25, pp. 409-425, Clarendon Press, Oxford.
12. Takemura, R. and Werb, Z. (1984) Am. J. Physiol. 246, C1-C9.
13. Nathan, C.F. (1987) J. Clin. Invest. 79, 319-326.
14. Moncada, S., Palmer, R.M.J. and Higgs, E.A. (1991) Pharmacol. Rev. 43, 109-142.
15. Beckmann, J.S., Beckmann, T.W., Chen, J., Marshall, P.A. and Freeman, B.A. (1990) Proc. Natl. Acad. Sci. USA 87, 1620-1624.
16. Neumann, H.A.M., Carlsson, K. and Brom, G.H.M. (1992) Eur. J. Clin. Pharmacol. 43, 423-426.
17. Hertog, K.G.L., Feskens, E.J.M., Hollman, P.C.H., Katan, M.B. and Kromhout, D. (1994) Nutr. Cancer 22, 175-184.

ere
1
the
ing

we
ious
n.
It is
ct of